HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF CEREAL-DERIVED CARBOHYDRATES

INTRODUCTION

During the last 20 years, High Performance Liquid Chromatography (HPLC) has developed from a mild curiosity into a powerful method for carbohydrate analysis. In this chapter, we will describe current state-of-the-art methods for HPLC analysis of cereal-derived carbohydrates. Separations of simple sugars, sugar alcohols, and cyclic- and linear- oligosaccharides up to a dp of approximately 85 will be described. High performance separations of polysaccharides will be covered in another chapter in this book. Some information will be given here on stationary phases, instruments, and other equipment useful for HPLC analyses. If more details in this area are required, the reader will be referred to a number of extensive recent reviews.

It is the aim of this chapter to present the most recent carbohydrate HPLC methods that are available and to indicate which of these methods are most frequently used for specific applications in carbohydrate-related industries. In order to obtain the latter information, a voluntary survey was completed by skilled practitioners of HPLC at 20 major companies in the wet milling, ingredient manufacturing, food, alcoholic- and non-alcoholic beverage, and candy manufacturing industries. Companies which participated are included in Table I.

INSTRUMENTATION

Instrumentation for carbohydrate HPLC analysis has recently been reviewed (Hicks, 1988) so only critical aspects will be discussed.

Solvent Delivery Systems

Almost all modern, commercially available HPLC solvent delivery systems are useable for carbohydrate analysis. However, since detectors commonly used with carbohydrate analyses are quite sensitive to changes in solvent flow, pressure, and composition, those pumping systems which provide the most pulse-free and precisely blended mobile phases are desirable. Most recent single- and multi-pump commercial systems can provide acceptable performance. Since HPLC detectors are also sensitive to dissolved gasses in mobile phases, the use of commercial degassing units or those systems which operate under a positive pressure of an inert gas such as helium are also recommended. Because many users of analytical HPLC techniques will want to scale up to preparative levels, it is recommended that researchers purchase HPLC systems capable of delivering flow rates useful for both analytical (0.1-5 ml/min) and preparative (5-50 ml/min) applications. Such systems which provide these flow rates without compromising performance are commercially available. Because many methods require the use of buffers and alkaline mobile phases, systems configured with inert non-metallic components, such as polyetheretherketone (PEEK), and self washing pump heads, can avoid corrosion of components and destruction of pump seals and pistons, saving considerable maintenance cost and time.

TABLE I
Participants of HPLC Survey

Type of company	Participants in Survey
Milling Companies	American Maize-Products Co.; A. E. Staley Manufacturing Co; Cargill Analytical Services; Corn Products, a Unit of CPC International, Inc.; 2 more requesting anonymity.
Alcoholic and Non-alcoholic Beverage Manufacturers	Miller Brewing Co.; The Seven Up Co.; Adolph Coors Co.; The Coca-Cola Company.
Food Ingredient Manufacturers	Biospherics Inc.; The NutraSweet Co.; Kelco, a Division of Merck & Co., Inc.; Suomen Xyrofin Oy, Quest International.
Food and Confectionery Manufacturers	Hershey Foods Corp.; Kraft General Foods, Inc. (2 responses); Nabisco Brands; 1 requesting anonymity

Fittings, Valves, Tubing, and Injectors

One of the most common causes of poor resolution in HPLC is a poorly "plumbed" system. Columns, injectors, and other components, connected with excess tubing and poorly constructed unions lead to band broadening and poor resolution in the final chromatogram. The use of PEEK tubing and plastic ferrules and nuts has greatly simplified the "plumbing" of HPLC instruments. These components, which operate well at pressures typically encountered in HPLC analyses, can easily be used to plumb a system that contains a minimum amount of dead-volume. Plastic fittings also provide "universal" connections for fittings from various manufacturers. However, some plastic fitting may fail at elevated temperatures.

Fixed loop injectors are the most useful type for HPLC analysis. When loops are properly overfilled, reproducibility of the injected amount is excellent and one injector can serve for both analytical and preparative applications by simply installing a loop with the desired volume (available from 5 microliters to over 10 ml in volume).

Filters and Pre- or Guard-columns

HPLC column life can be greatly extended by installing a pre-column between the injector and the analytical column. Those pre-columns that contain replaceable 0.2 micron filters and replaceable cartridge-type precolumns are especially convenient and effective. It is not easy to predict how often a pre-column should be changed. This is usually determined after acquiring some experience with the particular HPLC method being used. Build-up of sample particulate material on the frits/filters on a system will result in a gradual rise in pressure. Whenever pressure rises more than a few psi, the cause should be determined and corrected. Properly working systems should operate at a consistent pressure.

Column Hardware

The most useful feature developed in recent years are cartridge-type columns in which the stationary phase, housed in a glass, metal, or plastic cartridge is used in conjunction with a device which may provide radial or axial pressure, thereby eliminating voids which may develop in the packed stationary phase. Such configurations can greatly extend the useful "lifetime" of a stationary phase and

also result in a lower replacement cost (only the cartridge is replaced).

Detection Systems

A detailed analysis of HPLC detection systems has been published (Hicks, 1988). The most commonly used detectors for carbohydrate analysis are refractive index (RI) detectors which were used by 95% of our survey respondents. The sensitivity and characteristics of commonly used detectors are given in Table II. In general, one should use the most sensitive and stable detector available since this permits injection of small samples (and corresponding impurities), resulting in longer column life. The majority of survey respondents listed RI detectors as the most stable, sensitive, trouble free, and useful for quantitative purposes. Since homologous series of oligosaccharides all give approximately the same peak area per unit weight when RI detectors are used (Scobell et al., 1977), one can quantify a whole series of oligosaccharides by using only one pure oligosaccharide of that series as an external standard. Pulsed amperometric detectors (PAD) programmed with optimized settings (LaCourse and Johnson, 1991), give reproducible and predictable responses for simple sugars, di-, and trisaccharides (Paskach et al., 1991). For higher oligosaccharides, the situation is not so clear and researchers who use High Performance Anion Exchange (HPAE) chromatography columns for their unequalled ability to separate oligosaccharides up to an extremely high DP level, are frequently frustrated by their inability to quantify all the corresponding chromatographic peaks with the PAD. A number of investigators have studied this detection system in order to predict response factors for oligosaccharides for which standards are not available. Koizumi, et al. (1989 and 1991a), for instance, demonstrated that the PAD response for DP 2-13 malto-oligosaccharides increased directly with the number of HCOH groups per oligosaccharide. The relative detector response continued to increase with larger oligosaccharides (DP 14-17) but it was not quite proportional to the number of HCOH groups per molecule. Barsuhn and Kotarski (1991) using the same PAD detector settings, showed that the PAD response per micromole of malto-oligosaccharide steadily increased from DP 2-7.

TABLE II
Characteristics of Detectors Used for Carbohydrate HPLC

Feature	RI	PAD	UV
Detectability	"Universal"	"Selective"	"Selective"
Lower limit of detection	20-40 ng	10-30 ng	1-12 μg
Advantages	Predictable response; useful for quantitative analysis; stable; easy to maintain; non-destructive; analytical and preparative use.	Can be used with solvent gradients; sensitive; monitors only analyte of interest.	High sensitivity for some analytes; monitors only selected analytes.
Disadvantages	Sensitive to changes in temperature, pressure, and solvent; not useable with gradients.	Response factors are not well understood; Must have standards for all analytes; requires maintenance.	Poor sensitivity for many carbohydrates; many interfering compounds.

Ammeraal et al. (1991) observed that the malto-oligosaccharide response per micromole increased from DP 2 to -14. Beyond that point, response was variable, but the standards used contained more than one DP oligosaccharide. If pure oligosaccharides of higher DP values were available, this would facilitate quantification of these oligosaccharides by PAD. Unfortunately methods do not currently exist to isolate malto-oligosaccharides larger than DP 14-17 (Koizumi et al., 1991a).

HPAE methods provide excellent separations of oligosaccharides in part, because of the ability to apply mobile phase gradients to the column. However, the changing solvent composition effects the response of the PAD detector, further complicating the accurate quantification of eluting oligosaccharides. An innovative approach to compensate for these detector response variations was proposed by Larew and Johnson (1988), who sent the effluent from the HPAE column into an immobilized glucoamylase reactor, which completely hydrolyzed the separated oligosaccharides to produce glucose, prior to entering the PAD detector. By this method, a single calibration

curve for glucose based on peak area was used to accurately quantify each malto-oligosaccharide from DP 2-7.

Three other types of detectors, flame ionization, polarimetric, and light scattering (mass) detectors have recently been reviewed (Hicks, 1988). These detectors have a number of features that make them ideal for detection of carbohydrates. For some reason, they have not been extensively utilized by workers in the field.

Respondents to our survey indicated that the majority (95%) used refractive index detectors with 27% using amperometric types and 11% reporting the use of UV detectors at low wavelengths. Of these individuals, 80% chose modern RI detectors as being the most sensitive, with amperometric detectors being next in sensitivity which is in contrast to the literature values in Table II. Refractive index detectors were considered to be the most stable (92%); most quantitative (83%) and most trouble free (75%). The most trouble-prone was indicated by 71% of respondents to be amperometric detectors. Fortunately, newer versions of the PAD detectors, such as the pulsed electrochemical detector (PED), and those from at least two other manufacturers, are addressing some of these problems. The above discussion of carbohydrate amperometric detection was applicable to gold electrodes. Luo et al. (1991) compared gold, platinum, copper, nickel, silver and cobalt as materials for constant potential amperometric detection of glucose and found the copper electrodes had the best range of linear response, detection limits, and stability. The use of various types of electrochemical detectors is likely to become more reliable and commonplace in the cereal carbohydrate field.

HPLC STATIONARY- AND MOBILE PHASES FOR CARBOHYDRATE ANALYSIS

Because this area has been covered in detail in recent reviews (Hanai, 1986; Hicks, 1988; Ball, 1990; Churms, 1990; Ben-Bassat and Grushka, 1991) only information pertinent to cereal-derived carbohydrates will be given here. At present there are four main types of stationary phases for HPLC of carbohydrates. These are given in Table III.

Normal Phases

Polar amino-bonded silica gels were introduced in the mid-1970's (Conrad and Fallick, 1974; Linden and Lawhead, 1976; Schwarzenbach, 1976, and Rabel et al., 1976) and were

immediately applied to numerous carbohydrate separations. Initially, these columns were relatively unstable, but improvements in manufacturing technology have resulted in phases with very reasonable column "life" (from several months to a year, depending on applications). In addition to these amino-bonded phases, silica gels with covalently attached diol (Brons and Olieman, 1983), carbamoyl or polyamine (Koizumi et al., 1987 and 1991a) and cyclodextrin (Armstrong and Jin, 1989), functionalities have now been developed. Some early literature mistakenly described the use of these columns with acetonitrile/water mobile phases as "reversed-phase" chromatography, however, it is clearly a normal-phase system because increasing the amount of water, the "strong" solvent, in the mobile phase leads to decreased retention times for all sugars. These columns provide excellent separation of mono-, di- (Nikolov et al., 1985a), tri- (Nikolov et al., 1985b), and higher (Kainuma et al., 1981) oligosaccharides. In addition, their large sample capacity makes them quite useful for preparative applications (Hicks and Sondey, 1987).

TABLE III
Stationary Phases Used for HPLC of Neutral, Cereal-Derived Carbohydrates

Classification	Examples
Normal Phase	Silica gel with bonded aminopropyl-, diol-, or cyclodextrin groups; organic polymer-bound amino functionality.
Reversed-phase	Silica gel alkylated with C_8,C_{18},or Phenyl groups; graphite; polystyrene-divinylbenzene-based polymers.
Cation Exchange Phases	Sulfonated polystyrene-divinylbenzene polymers in H^+, Ca^{++}, Pb^{++}, Ag^+, etc. form.
Anion Exchange Phases	Pellicular, sulfonated polystyrene-divinylbenzene strongly basic anion exchange resins.

Their disadvantages include: the need for expensive and thoroughly-degassed solvent (acetonitrile), shorter lifetime than ion exchange columns (see section below), and the insolubility of larger oligosaccharides in the mobile phase. Despite these factors, almost half (47%) of survey respondents reported using these phases. Further details about these phases are given in the reviews noted above.

Reversed-Phases

These alkylated silica gels are extremely useful in other areas of chemistry but receive minor use by carbohydrate analysts (32% of survey respondents). Underivatived mono- and di-saccharides have very little retention on these columns and reducing oligosaccharides generally elute in anomeric pairs. Situations in which these phases may be useful for carbohydrate separations may be found in articles by Vratny et al., (1983), Porsch (1985), McGinnis, et al., (1986) and Hicks (1988), in which their uses for separating mono-, linear-, and cyclic oligosaccharides as well as sugar derivatives are described. The most used phase of this type, C_{18} bonded silica gel, is usually eluted with pure water, or water containing 5-10% methanol. An innovative aminopropyl "doped" reversed-phase material that provided excellent separation of oligosaccharides was developed by Porsch (1985) but unfortunately was never made commercially available.

Cation Exchange Resin Phases

Since the late 1970's, these phases have been the "workhorse" of the analytical carbohydrate chemist. This was confirmed by our survey in which 95% of the respondents used such columns. This is not surprising since they are not only versatile, but durable (some have lasted for up to 10 years, with only occasional regeneration). Such phases, in a variety of ionic forms, including Ag^+, Ca^{++}, Pb^{++}, Na^+, and H^+, can be used with a simple mobile phase of water (or dilute acid for the H^+-form) for the separation of practically every class of carbohydrate. In the survey, the frequency of use was $Ca^{++} > Ag^+ > H^+ > Pb^{++} > Na^+$-forms. These columns are usually run at elevated temperatures (70-85°C) to prevent the separation of anomeric forms of reducing sugars. The historical advances which led to the commercialization of these phases has been described elsewhere (Hicks, 1988) but it is important to note that researchers in the corn refining industry played a major role in these developments (see Brobst et al., 1973; Scobell et al., 1977; Fitt, 1978; Fitt et al., 1980; and Scobell and Brobst, 1981). For information on the care, handling, mechanisms of separation, and general applications, see Hicks (1988).

Anion Exchange Resin Phases

Since their introduction (Rocklin and Pohl, 1983), pellicular, high-performance anion exchange resin phases have steadily gained popularity. About 32% of our survey respondents use them. When they are used with alkaline eluents (pH 10-12), sugars act as anions and separate according to their pKa values and additional steric factors, resulting in powerful separation capability for numerous sugars, small oligosaccharides and sugar alcohols (Paskach et al., 1991; van Riel and Olieman, 1991) and starch-derived oligosaccharides as large as DP 85 (Koizumi et al., 1989; Koizumi et al., 1991a; Ammeraal et al., 1991). No other HPLC stationary phase can match the versatility, selectivity, and efficiency of these relatively new pellicular resins.

In our survey, respondents ranked the following phases as the most durable: cation exchange resins (67%), high-performance anion exchange resins (17%), reversed-phases (8%) and polar amino-bonded phases (8%). The low durability ranking of high-performance anion exchange resins may be misleading. Most investigators have used these phases for a short period, and in many cases have not yet learned how to effectively prolong the "life" of the column. In the laboratory of one of the authors, a high-performance anion-exchange column (Dionex HPIC-AS6) has been used for 4 years with little change in selectivity and resolution. When resolution begins to fail, the column is washed with 3 M NaOH solution, which cleans the phase and restores nearly original performance.

METHOD SELECTION FOR SPECIFIC APPLICATIONS

In our survey we asked respondents to give the source of the HPLC methods used in their laboratories. The results were: 37% from the scientific literature, 33% developed at their location, and 30% official methods from a group such as AOAC, CRA, AACC, etc. It was interesting to note that less than one third of the respondents used officially sanctioned HPLC methods for carbohydrate analysis, even though a number of these methods are available. For instance, the 1990 edition of the AOAC Official Methods of Analysis provides official HPLC methods for major saccharides in corn syrup (method 979.23), minor saccharides in corn sugar (983.22), sugars in milk chocolate (980.13), and sugars in pre-sweetened cereals (982.14). The AACC has two official HPLC methods, 80-04 for simple sugars in cereal products, and 80-05 for saccharides in corn syrup. The

sixth edition of the Corn Refiner's Association's Standard Analytical Methods manual lists HPLC method E-61 for saccharides in corn syrup, and F-51 for minor saccharides in corn sugar.

Survey respondents were also asked to provide specific stationary phases, mobile phases, and detectors they used for separating specific carbohydrates. The data were collected, analyzed and are given in Table IV. Most of the methods given by respondents were readily identified with known literature references, which are given in the Table. In most cases, original references are given for a particular separation, rather than the most recent reference that simply uses that technique. This was done because such early references generally have a wealth of information that is not always conveyed in later publications. The original references of Scobell et al. (1977) and Scobell and Brobst (1981) are given for separation of dextrose, maltodextrins, corn syrup solids, HFCS, and fructose because those references provide details of resin conversion, packing, effects of different metal ion ligands, effects of temperature, and other fundamental parameters on sugar separations. More recent references such as that of Warthesen (1984) are given to indicate more recent refinements of methods and to indicate sources of commercially available columns that are pre-packed with resins of optimum size and counter ion for specific applications.

TABLE IV

Survey Results: Methods Used for Specific Applications

Carbohydrate	Stationary Phase	Eluent/Detector	Reference
dextrose:	43% Ca^{++}-CER[a]	H_2O, 85°, RI	Scobell et al., 1977
	21% NH_2 phase[b]	CH_3CN/H_2O, RI	Verhaar and Kuster, 1981
	7% Ag^{+}-CER	H_2O, 85°, RI	Scobell and Brobst, 1981
	7% Na^{+}-CER	.001 M Na_2SO_4, RI	
	7% HPAE[c]	NaOH, PAD, RI	Paskach et al., 1991
other monosacch's:	50% Ca^{++}-CER	H_2O, RI	Richmond et al., 1983
	21% HPAE	NaOH, PAD	Paskach et al., 1991
	14% Pb^{++}-CER	H_2O, RI	Petersen et al. 1984
	14% NH_2 phase	CH_3CN/H_2O, RI	Verhaar and Kuster, 1981
malto-dextrins:	56% Ag^{+}-CER	H_2O, RI	Wartheesen, 1984
	22% NH_2 phase	CH_3CN/H_2O, RI	Kainuma et al., 1981
	11% Ca^{++}-CER	H_2O, RI	Scobell and Brobst, 1981
	11% HPAE	NaOH/NaOAc, PAD	Koizumi et al., 1989
			Ammeraal et al., 1991
di- and	31% NH_2 phase	CH_3CN/H_2O, RI	Nikolov et al., 1985a, 1985b
tri-sacch's:	23% HPAE	NaOH/NaAc, PAD	Paskach et al., 1991
	15% RP[d]	H_2O, RI	Rajakyla, 1986
	15% Ag^{+}-CER	H_2O, RI	van Riehl and Olieman, 1986
	8% Ca^{++}-CER	H_2O, RI	Brons and Olieman, 1983
	8% Pb^{++}-CER	H_2O, RI	Voragen et al., 1986
corn syrup solids:	36% Ca^{++}-CER	H_2O, RI	Scobell and Brobst, 1981
	27% Ag^{+}-CER	H_2O, RI	Wartheesen, 1984; Scobell and Brobst, 1981.
	9% Pb^{++}-CER	H_2O, RI	Voragen et al., 1986
	9% Na^{+}-CER	.001M Na_2SO_4, RI	
	9% NH_2 phase	CH_3CN/H_2O, RI	Kainuma et al., 1981
	9% HPAE	NaOH/NaAc, PAD	Koizumi et al., 1989
HFCS:	67% Ca^{++}-CER	H_2O, RI	Scobell, et al., 1977
	22% NH_2 phase	CH_3CN/H_2O, RI	Verhaar and Kuster, 1981
	11% HPAE	NaOH, PAD	

TABLE IV, Continued
Survey Results: Methods Used for Specific Applications

Carbohydrate	Stationary Phase	Eluent/Detector	Reference
fructose:	44% Ca^{++}-CER	H_2O, RI	Scobell et al., 1977
	33% NH_2 phase	CH_3CN/H_2O, RI	Iverson and Bueno, 1981
	11% Na^+-CER	.001M Na_2SO_4, RI	
	11% HPAE	NaOH, PAD	Rocklin and Pohl, 1983
Poly-dextrose®:	50% HPGPC[e]	NaAc buffer, RI	
	50% Na^+-CER	H_2O, RI	
pyro-dextrins:	50% Ag^+-CER	H_2O, RI	
	50% HPGPC	H_2O, RI	
non-starch syrups:	20% Ca^{++}-CER	H_2O, RI	Schmidt et al., 1981
	20% Ag^+-CER	H_2O, RI	Wood et al., 1991
	20% RP	H_2O, RI	Vratny et al., 1983
	20% NH_2 phase	CH_3CN/H_2O, RI	Smiley et al., 1982
	20% HPAE	NaAc/NaOH, PAD	Ammeraal et al., 1991
cyclo-dextrins:	50% Ca^{++}-CER	H_2O, RI	Hokse, 1980
	50% other		Koizumi et al., 1988 and 1991b
sugar alcohols:	80% Ca^{++}-CER	H_2O, RI	Wood et al., 1980
	20% HPAE	NaOH, RI,PAD	Paskach et al., 1991
hydro-genated corn syrup:	50% Ca^{++}-CER	H_2O, RI	
	50% Ag^+-CER	H_2O, RI	
Sucralose:	100% RP	25% CH_3CN/ H_2O pH 2.7, RI	
Saccharides in wort and beer	NH_2-phase	CH_3CN/H_2O, RI	Uchida et al., 1990

[a]43% Ca^{++}-CER = 43% of respondents used a Ca^{++}-form Cation Exchange Resin for this application.
[b]Polar, amino-type normal phase, such as aminopropyl silica gel.
[c]High performance anion exchange resin.
[d]Reversed-phase, such as C_{18}.
[e]High-performance gel permeation chromatography.

In addition to the information in Table IV for carbohydrate-specific methods, it may be useful to provide some general guidelines on method selection. These are given below.

General Guidelines for HPLC Method Selection

1. Use the simplest method that provides an acceptable separation.
2. Use cation exchange resin-based phases whenever possible:

For analysis of:	First try:
- one or two monosaccharides	Ca^{++}-form resins
- mixtures of several monosaccharides	Pb^{++}-form resins
- one or two disaccharides	Ca^{++}-form resins
- mixtures of several disaccharides	Ca^{++}- or Pb^{++}-form resins
- mixtures of mono-, di-, and higher oligosacch's	Ag^{+}-form resins
- mixtures of sugar alcohols	Ca^{++}-form resins.
- cyclodextrins	Ca^{++}-form resins

3. When choosing a cation exchange resin phase, use 8% crosslinked resins for monosaccharides and 4% crosslinked resins for di- and larger oligosaccharides. For separation of larger than DP-8 and less than DP-13 oligosaccharides, 2% crosslinked resins may be used (Hicks and Hotchkiss, 1988)

4. If cation exchange resin-based phases will not provide the needed separations, use a pellicular, high performance anion exchange (HPAE) phase.

5. If an HPAE phase is not effective, or is not available, try a normal phase column with an acetonitrile/water mobile phase. In selecting a normal phase column, the use of cyclodextrin-bonded phase (Armstrong and Jin, 1989) and diol-bonded phase (Brons and Olieman, 1983) columns should be examined as well as amino-bonded phases because of the durability of the former two.

6. Use RI detection whenever possible for isocratic separations. If it is not possible to use RI detection (gradient separations, etc.), use a UV detector if an appropriate UV absorbing group is present on the analyte. Otherwise, use a pulsed amperometric detector if available.

7. Consider using an evaporative light scattering ("mass") or flame ionization detector if available. Unlike RI detectors, these can accommodate solvent gradients.

8. Always use pre-filters and pre-columns containing a stationary phase similar to that in the analytical column.

9. Use mobile phase to dissolve samples prior to injection. This may not be necessary with HPAE-PAD, (Paskach et al., 1991).

TECHNOLOGY BREAKTHROUGHS

Survey participants were asked to list the recent techniques, stationary phases, detectors, etc. that have had the greatest positive impact on their ability to analyze carbohydrates. Those breakthroughs are listed in Table V. The most important breakthrough was found to be the development of recent RI detectors which are capable of providing extremely sensitive and stable detection of carbohydrates. These detectors were first introduced in the mid 1980's and are now available from at least six manufacturers. These detectors are commonly used with the second ranked breakthrough, the cation exchange resin based stationary phases, which generally use pure water as a mobile phase. This simple eluent also contributes significantly to the sensitivity obtained on these detectors. The third ranking breakthrough, high-performance anion exchange chromatography, coupled with pulsed amperometric detection, is a relatively new technique, and may eventually replace many of the currently popular HPLC methods. Conversely, the fourth ranked breakthrough, the development of aminopropyl bonded silica gel stationary phases would have ranked much higher in surveys 5 to 10 years ago and may continue to receive less attention in the coming years.

TABLE V
Greatest Technology Breakthroughs in HPLC of Carbohydrates

Rank (% of responses)	Breakthrough
1 (41%)	Ultra-sensitive and -stable RI detectors
2 (35%)	Cation exchange resin-based HPLC columns
3 (18%)	High-performance anion exchange columns coupled with pulsed amperometric detection
4 (6%)	Aminopropyl silica gel bonded "carbohydrate" HPLC columns

PREPARATIVE HPLC OF CEREAL-DERIVED CARBOHYDRATES

The combination of relatively large sample injections and non-destructive detection makes HPLC an ideal preparative technique. During the late 1970's and early 1980's, commercial preparative HPLC systems were introduced. These initial systems were far from ideal since they operated at extremely high flow rates and therefore, used large volumes of solvents. The preparative columns available were packed with relatively inefficient particles, leading to poor resolution. In many cases, better, and certainly less expensive results were obtained on low-pressure LC equipment than on those "first generation" preparative HPLC instruments. Currently, however, highly advanced systems are available which consist of automated, microprocessor controlled solvent delivery systems, extremely efficient columns, and "intelligent" fraction collectors which can be programmed to collect using a drop, time, or peak mode. Such systems have been used for the efficient preparative isolation of mono- and di-saccharides (Hicks et al., 1983) and malto-oligosaccharides (Hicks and Sondey, 1987). For a complete review of preparative HPLC of carbohydrates, please see Hicks (1988).

CURRENT NEEDS AND POTENTIAL SOLUTIONS

Survey respondents provided a list of several important needs in the HPLC area. Some of these and some potential solutions are given below.

Need: Ability to quantify lactose in the presence of fructose/sucrose/maltose.

Solution: Relatively recent methods are available for this separation using cation exchange resin- (van Riel and Olieman, 1986) and high-performance anion exchange resin- (van Riel and Olieman, 1991) phases.

Need: Ability to separate mixtures of saccharides of the same DP value.

Solution: Separation on high-performance anion exchange resin phases (Paskach et al., 1991; Koizumi et al., 1989).

Need: Need more effective methods to analyze Polydextrose®.

Solution: When Polydextrose® is present in matrices free from other larger oligosaccharides, a method based on a Ca^{++}-form cation exchange resin phase may be appropriate (Noffsinger et al., 1990). Alternatively, HPAE-PAD, with its excellent selectivity toward oligosaccharides should be applied to this analysis.

Need: Need methods to quantify higher oligosaccharides (>DP 20) in mixtures.

Solution: For malto-oligosaccharides for which no standards exist, use of an immobilized glucoamylase reactor (Larew and Johnson, 1988), prior to the detector, can provide quantitative information.

CONCLUSIONS

HPLC methods exist for analysis of most cereal-derived carbohydrates. Breakthroughs in technology over the last 10-15 years have resulted in methods which allow accurate analysis of mixtures that were impossible to analyze a generation ago. Technology continues to develop in this field and additional breakthroughs in separation and detection methods will likely continue. Industrial laboratories surveyed in this work had a relatively sophisticated working knowledge of the state-of-the-art in

HPLC methods and used them as their methods of choice for numerous analyses of cereal-derived carbohydrates.

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